



DOCKET NO: 217865US0PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF

HARUJI SAWADA, ET AL.

: EXAMINER: H. LILLING

SERIAL NO: 10/031,569

FILED: JANUARY 22, 2002

: GROUP ART UNIT: 1651

FOR: CHOLESTEROL-LOWERING
AGENTS, SECONDARY BILE ACID
PRODUCTION INHIBITORS AND FOODS
AND DRINKS

DECLARATION UNDER 37 C.F.R. § 1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

Now comes Yasuto Yoshida who deposes and
states:

1. That I am a graduate of Tokyo University of Science and received a
MS degree in the year 1994.
2. That I have been employed by Yakult Honsha Co., Ltd. for 11 years as a
researcher in the field of Applied Microbiology.
3. That I understand the English language or, at least, that the contents of the
Declaration were made clear to me prior to executing the same.
4. That the following experiments were carried out by me or under my direct
supervision and control. These experiments show the amount of *Kluyveromyces* required to
lower cholesterol levels.

5. As shown in Yoshida et al., Biosci. Biotechno. Biochem. 68:1185 (2004), the minimum quantity of *Kluyveromyces marxianus* YIOT8292 necessary to manifest a significant reduction in cholesterol level in male Wistar Rats is at least 3.0% (w/w) in a food composition.

6. A yeast content below 3.0% (w/w) did not bring about a reduction in cholesterol level, see Yoshida et al., page 1189, Experiment 3, and page 1190, Fig. 1.

7. As shown by the attached Experimental Report "Study on minimum effective amount of cell wall fraction (KM-CW) for *Kluyveromyces marxianus* YIT 8292", the minimum quantity of *Kluyveromyces marxianus* necessary to reduce plasma cholesterol level in patients having mild hypercholesterolemia was 2 g/day, when a tablet of KM-CW was administered, see 3-1-2 and Figs. 2-3. Taking into account that the cell wall (CW) accounts for about 70% of the whole yeast cell, one would need to take at least 2.86 g/day of whole yeast cells in order to provide this cholesterol reducing effect.

8. The amount of yeast contained in the food composition of Muys, U.S. Patent No. 3,995,066, is less than that required to reduce cholesterol levels. Muys discloses that the mass of haploid yeast and diploid yeast is 15×10^{-12} g/cell, respectively, see page 11, Table V.

9. The yeast content in the food composition of Muys ranges from 10^3 - 10^7 cells/g emulsion as described in the Abstract and in Claim 1.

10. Converting this yeast content into mass%, the following results are obtained:

haploid yeast	0.0000012-0.015% (w/w)
diploid yeast	0.0000020-0.020% (w/w).

11. As shown above, one needs to consume a food composition having at least 3% (w/w) yeast to reduce cholesterol. However, 3% is over 150 times more than the yeast

content in the Muys food composition. Therefore, the Muys food composition does not contain enough yeast to reduce the level of cholesterol.

12. If one intends to take 2.86 g/day of yeast as described above as necessary to reduce the cholesterol level, then one must consume about 14.3 (diploid yeast) or about 19.1 kg (haploid yeast) of the Muys food composition. Such large quantities of food could not effectively be administered to a subject.

13. The undersigned petitioner declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

14. Further deponent saith not.

Signature: _____

Yasuto Yoshida

Date _____

November 18, 2005.